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Datasheet for the decision of 25 January 2021

Case Number: T 0159/17 - 3.3.08

Application Number: 09818482.3

Publication Number: 2346994

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Language of the proceedings: EN

Title of invention:
NON-HUMAN MAMMALS FOR THE PRODUCTION OF CHIMERIC ANTIBODIES

Applicant:
Ablexis, LLC

Headword:
Chimeric antibodies/ABLEXIS

Relevant legal provisions:
EPC Art. 54, 56, 84, 83, 123(2)

Keyword:
Main request - requirements of the EPC fulfilled (yes)

Decisions cited:
T 0578/06, T 0716/08

Catchword:

Europäisches Patentamt
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Beschwerdekammern

Boards of Appeal

Chambres de recours

Boards of Appeal of the
European Patent Office
Richard-Reitzner-Allee 8
85540 Haar
GERMANY
Tel. +49 (0)89 2399-0
Fax +49 (0)89 2399-4465

Case Number: T 0159/17 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 25 January 2021

Appellant: (Applicant)	Ablexis, LLC 10581 Roselle Street, Suite 115 San Diego, CA 92121 (US)
Representative:	Kirchhofer, Natalie Cohausz & Florack Patent- & Rechtsanwälte Partnerschaftsgesellschaft mbB Bleichstraße 14 40211 Düsseldorf (DE)
Decision under appeal:	**Decision of the Examining Division of the European Patent Office posted on 17 June 2016 refusing European patent application No. 09818482.3 pursuant to Article 97(2) EPC.**

Composition of the Board:

Chairman	B. Stolz
Members:	M. R. Vega Laso
	A. Bacchin

Summary of Facts and Submissions

I. The appeal of the applicant (appellant) lies from a decision of an examining division posted on 17 June 2016, refusing the European patent application No. 09818482.3 with the title "Non-human mammals for the production of chimeric antibodies". The application was filed under the Patent Cooperation Treaty and published as WO 2010/039900 (in the following "the application as filed").

II. In the decision under appeal, the examining division found that the subject-matter of the claims according to each of the main request and the auxiliary requests 1 to 5 then on file lacked an inventive step (Article 56 EPC) because, in their view, there was no evidence in the application as filed that the purported technical effect is in fact achieved and the objective technical problem solved by the claimed subject-matter. Moreover, claim 6 of the main request then on file was considered to lack clarity (Article 84 EPC).

III. Together with its statement of grounds of appeal, the appellant re-filed the sets of claims according to the auxiliary requests 1 to 5 underlying the decision under appeal as, respectively, main request and auxiliary request 1 to 4 in appeal proceedings. The appellant also submitted experimental data (Annexes A and B) and documentary evidence (Annex C), and requested oral proceedings as a subsidiary request.

IV. Pursuant to its request, the appellant was summoned to oral proceedings before the board.

V. In a communication sent in preparation of the oral proceedings, the board expressed a provisional opinion on the probative value of the experimental evidence submitted by the appellant, and raised new objections under Articles 123(2) and 84 EPC.

VI. The appellant replied to the board's communication. It filed ten sets of amended claims as new auxiliary requests 1 to 10, and re-numbered its previous auxiliary requests 1 to 4 as auxiliary requests 11 to 14.

VII. Oral proceedings were held on 25 January 2021. At the outset of the oral proceedings, the appellant withdrew its main request and re-filed the previous auxiliary request 1 as its new main request.

VIII. Independent claims 1, 6, 7 and 9 according to the main request read as follows:

"1. A homologous recombination competent mouse cell having a genome comprising (1) a human VH gene segment and (2) a portion of a syngeneic Ig heavy chain locus, comprising
(a) a part of mouse gene segments downstream of JH,
(b) a syngeneic CH1 domain replaced with a human CH1 domain,
(c) a human upper hinge gene segment,
(d) a mouse CH2 domain and a mouse CH3 domain,
and
wherein said human VH gene segment and said syngeneic Ig heavy chain locus replace all or a portion of an endogenous Ig heavy chain locus, thereby resulting in a chimeric Ig heavy chain locus capable of undergoing gene rearrangement and thereby producing a diversified

repertoire of chimeric antibodies such that said cell comprises a genome encoding a chimeric Ig heavy chain.

6. A method of producing the homologous recombination competent mouse cell according to any one of claims 1-5 comprising the steps of:
producing a first BAC comprising a human VH gene segment;
producing a second BAC comprising a portion of a syngeneic Ig heavy chain locus, comprising
(a) a part of mouse gene segments downstream of JH,
(b) a syngeneic CH1 domain replaced with a human CH1 domain,
(c) a human upper hinge gene segment,
(d) a mouse CH2 domain and a mouse CHS domain; and
introducing said first BAC into a homologous recombination competent mouse cell and replacing all or a portion of an endogenous VH gene segment via homologous recombination; and
introducing said second BAC into said cell and replacing all or a portion of an endogenous Ig heavy chain locus via homologous recombination, wherein said cell comprises a genome encoding a chimeric Ig heavy chain.

7. A knock-in mouse having a genome comprising (1) a human VH gene segment and (2) a portion of a syngeneic Ig heavy chain locus, comprising
(a) a part of mouse gene segments downstream of JH,
(b) a syngeneic CH1 domain replaced with a human CH1 domain,
(c) a human upper hinge gene segment,
(d) a mouse CH2 domain and a mouse CH3 domain,
wherein said human VH gene segment and said syngeneic Ig heavy chain locus replace all or a portion of an endogenous Ig heavy chain locus, thereby resulting in a

chimeric Ig heavy chain locus capable of undergoing gene rearrangement and thereby producing a diversified repertoire of chimeric antibodies, such that said mouse comprises a genome encoding a chimeric Ig heavy chain.

9. A chimeric antibody comprising a human VH, a human CH1 domain, a human upper hinge region, a mouse CH2 domain and a mouse CH3 domain."

Dependent claims 2 to 5 are directed to various embodiments of the cell of claim 1. Dependent claim 8 is directed to an embodiment of the knock-in mouse of claim 7. Dependent claim 10 concerns an embodiment of the antibody of claim 9. Claims 11 to 13 are directed to, respectively, a use of the mouse of claim 7 or 8, a pharmaceutical composition comprising the antibody of claim 9 or 10, and the chimeric antibody of claim 9 or 10 for use in therapy.

IX. In the present decision, reference is made to the following documents:

(4): WO 02/066630 A1, published on 29 August 2002;

(5): US 2007/0009957 A1, published on 11 January 2007;

(6): WO 2006/117699 A2, published on 9 November 2006;

(10): M. Torres *et al.*, 4 May 2007, Journal of Biological Chemistry, Vol. 282, No. 18, pages 13917 to 13927;

(11): M. Torres *et al.*, December 2007, PLoS ONE, Issue 12, e1310; and

Annexes A and B submitted on 27 October 2016.

X. The submissions made by the appellant were essentially as follows:

Admission of the main request into the proceedings

The amended claims according to the present main request were derived from those of the auxiliary request 1 underlying the decision under appeal. The amendments had been made in direct response to objections concerning Articles 123(2), 84 and 56 EPC which had been raised for the first time in the communication issued by the board in preparation of the oral proceedings. Since the amended claims could not have been filed either in examination proceedings or earlier in appeal proceedings, they should be admitted and considered by the board.

Articles 123(2) and 84 EPC

The amendments introduced into the claims overcame the objections raised by the board in its communication and complied with Articles 123(2) and 84 EPC. Figures 4A, 4B and 4C in the application as filed depicted the replacement of fully murine constant regions (indicated as white boxes) with **chimeric** constant regions Cμ, Cδ, Cγ1-3 (indicated as boxes with partly hashed and partly white filling). Each of these constant region "boxes" stood for the exons coding for the CH1, hinge, CH2 and CH3 domains of each antibody isotype heavy chain. The differential shading of the Cμ, Cδ, Cγ1-3 "boxes" in the bottom parts of Fig. 4B and in Fig. 4C as comprising human sequences in the 5' (left, hashed shading) and murine sequences in the 3' (right, white shading) portions of the respective C-regions clearly depicted, when read together with the remaining

disclosure in the application as filed (see claim 1 as originally filed) that the 5' parts of the C-regions (i.e. CH" and CH3) remain murine sequences.

Article 56 EPC

The experimental data in Annexes A and B showed that antibodies produced by mouse cells and mice according to the claimed invention were effective and had the same binding kinetics and affinity as well as the same potency as fully human antibodies derived therefrom. Thus, the technical effect of the claimed subject-matter had been substantiated. Contrary to the findings in the decision under appeal, the claimed subject-matter did not represent an arbitrary solution devoid of any inventive merit, but a purposive improvement involving an inventive step.

XI. The appellant requested that the decision under appeal be set aside and a patent be granted on the basis of the claims of the main request filed at the oral proceedings on 25 January 2021.

Reasons for the Decision

Article 123(2) EPC

1. The present main request is derived from the auxiliary request 1 underlying the decision under appeal. The examining division acknowledged that the claimed subject-matter had a basis in the application as filed, and pointed in particular to Figures 4A, 4B and 4C, and the passage on page 27, lines 9 to 12 of the application (see first two paragraphs under the heading

"Auxiliary Request 1" on page 21 of the decision under appeal).

2. The board is satisfied that the subject-matter of the amended claims according to the present main request does not extend beyond the content of the application as filed (Article 123(2) EPC). A mouse cell as defined in amended claim 1 has a basis in claims 1, 8 and 14 combined with the disclosure in the passage on page 27, lines 9 to 12 of the application as filed. Basis for dependent claims 2 to 5 is found in, respectively, claims 3, 2, 4 and 13 of the application as filed.

3. The method of independent claim 6 has a basis in claims 15 and 28 of the application as filed.

4. Basis for the knock-in mouse of independent claim 7 is found in claims 77, 85 and 105 combined with the disclosure in the passage on page 27, lines 9 to 12 of the application as filed. Dependent claim 8 is based on claim 79 of the application as filed.

5. Chimeric antibodies according to claims 9 and 10 have a basis in claim 106 of the application as filed which refers to, *inter alia*, claims 77, 85 and 105.

6. Basis for claim 11 is found in claim 107 of the application as filed. Claims 12 and 13 have a basis in, respectively, claim 118 and the passage on page 39, lines 4 and 5 of the application as filed.

7. No objections under Article 84 EPC concerning the auxiliary request 1 then on file were raised in the decision under appeal. In view of the amendments introduced into the claims, the board is satisfied that the requirements of Article 84 EPC are fulfilled.

Articles 83 and 54 EPC

8. In the decision under appeal, the examining division did not raise any objections of lack of sufficient disclosure, and acknowledged novelty over document (4) (see second paragraph from the bottom of page 21 of the decision under appeal). In view of the documents on file, the board sees no reason to raise any objections in this respect of its own motion.

Article 56 EPC

9. In the decision under appeal, document (4) was considered to be the closest state of the art for the subject-matter of the auxiliary request 1 then on file. In the examining division's view, the passage from page 42, line 20 to page 44, line 30 of document (4) clearly emphasized that fully human antibodies are not optimal, and that murine Fc regions are crucial for maturation and affinity of chimeric antibodies comprising a humanized variable region. However, the examining division acknowledged that neither the passage in question nor document (4) as a whole suggested or showed that antibodies with a constant region comprising a human CH1-upper hinge-middle hinge segment combined with murine CH2 and CH3 regions would be effective "*... and provide chimeric antibodies with optimal characteristics*" (see third paragraph on page 23 of the decision under appeal).

10. In the board's view, the same applies with respect to chimeric antibodies produced by a cell as defined in present claim 1, the constant region of which comprises a human CH1-upper hinge segment combined with murine CH2 and CH3 regions.

11. In the passage from page 43, line 27 to page 44, line 3 of document (4), it is stated that:

> "*The mouse will create antibodies that are human (VDJ/VJ)-mouse constant region, which will have the following benefits over the previously available HuMAb mice that produce totally human antibodies. Antibodies generated by the new mouse will retain murine Fc regions which will interact more efficiently with the other components of the mouse B cell receptor complex, including the signalling components required for appropriate B cell differentiation (such as Iga and Igb). Additionally, the murine Fc regions will be more specific than human Fc regions in their interactions with Fc receptors on mouse cells, complement molecules, etc. These interactions are important for a strong and specific immune response, for the proliferation and maturation of B cells, and for the affinity maturation of antibodies*".

12. As apparent from this passage, the mouse described in document (4) has in its genome a chimeric locus that includes gene segments encoding the human variable region and the (complete) murine constant region. However, document (4) does not teach or even suggest a mouse having a chimeric locus as defined in claim 1 in which part of the constant region, namely the CH1 domain and the upper hinge region are human, while the CH2 and CH3 domains remain murine.

13. According to the present application, the technical effect associated with this difference is that the "*... antibodies produced by the knock-in animals of the*

present invention do not exhibit the reduction or loss of activity and potency seen in antibodies from other chimeric antibody producing animals when the human V region is appended to a human C region to make a fully human antibody ..." (see page 19, lines 25 to 28 of the application as filed).

14. In view of these statements, the examining division formulated the objective technical problem to be solved starting from document (4) as the provision of "*... means of generating in transgenic mouse, in response to an undefined antigen, chimeric humanized antibodies that* [, when further humanized,] *would retain affinity and specificity without eliciting an immune response*" (see page 22, first paragraph of the decision).

15. The examining division found that, since the purported technical effect had not been demonstrated, the problem of retaining affinity when the generated chimeric antibodies are further humanized could not be considered to have been solved. Consequently, "*... the (speculative) inclusion of human CH1, upper and middle hinge gene segments into the gene locus is just a design option that the skilled person may contemplate when trying to further humanize the chimeric constant region ...*" (see fourth paragraph on page 23 of the decision under appeal).

16. It is a fact that, while the examples of the present application describe engineering a murine Ig loci by incorporation of large bacterial artificial chromosomes (BACs) into embryonic stem cells in order to generate chimeric antibodies comprising a human CH1-upper hinge domain and murine CH2 and CH3 domains (see e.g. Example 8), there is no experimental evidence in the

application that the generated antibodies, when further humanized, do not suffer from a reduction or loss of activity and potency.

17. However, the disclosure of experimental data or results in the application as filed, while highly desirable, is not always required to establish that the claimed subject-matter solves the objective technical problem, in particular in the absence of substantiated doubts (see decision T 578/06 of 29 June 2011, points 13 to 15 of the Reasons). According to the established jurisprudence of the boards of appeal, the assessment of inventive step is to be made on the basis of the information in the application as filed, and on account of the common general knowledge available to the skilled person at the effective date of the application. Experimental evidence submitted after the filing date to support that the claimed subject-matter solves the problem, can be taken into account if it is already credible from the disclosure in the application as filed that the problem is indeed solved (see, *inter alia*, decisions T 578/06, *supra*, and T 716/08 of 19 August 2010).

18. In the present case, the examining division did not substantiate its doubts about the suitability of the claimed invention to solve the formulated technical problem. The board sees no reason to consider the purported technical effect underlying the present invention as merely speculative, because it is not implausible, in the light of the common general knowledge available to the person skilled in the art at the effective date, that chimeric antibodies comprising a human CH1-upper hinge domain and murine CH2 and CH3 domains, when further humanized, may retain their activity and potency.

19. Under these circumstances, the experimental data submitted by the appellant as Annexes A and B to its statement of grounds of appeal can be accepted as evidence for a technical effect associated with the combination in the chimeric locus of a human CH1-upper hinge gene segment and a murine CH2-CH3 gene segment.

20. As apparent from Annex A, an antibody specific for human CD115 generated from a hybridoma obtained by immunizing transgenic mice harbouring in their genome a chimeric locus as defined in claim 1 (human V-D-J-CH1-upper hinge and murine middle hinge-CH2-CH3), when converted to a fully human antibody of three different isotypes (IgG1, IgG2 and IgG4), binds to CD115 with the same affinity as the chimeric human-murine antibody (see Table 1) and exhibits potency identical to the parental chimeric human-mouse antibody (see Table 2). Similarly, chimeric anti-IL-8 antibodies generated by mice having a chimeric locus as defined in claim 1, when fully humanized, retain the same affinity as the parental antibody (see Table 3 in Annex B).

21. In view of this experimental evidence, the board acknowledges that the objective technical problem as defined in the decision under appeal (see paragraph 14 above) is solved by the claimed invention.

22. Hence, the sole issue that remains to be decided is whether or not, starting from the chimeric locus having a human variable region and a murine constant region described in document (4), it was obvious to a person skilled in the art seeking to generate fully human antibodies that retain the affinity and potency of the chimeric parental antibodies, to modify the locus by

replacing the murine CH1 and upper hinge gene segment with its human counterpart.

23. Document (6) addresses the same problem as the present invention, namely the decrease in affinity or other characteristics when the chimeric antibodies produced in transgenic mice are humanized (see paragraph [0011]). The solution proposed in document (6) is to replace the murine sequences that encode one or more of the murine heavy chain constant regions (e.g. the murine α region, the murine Cγ3, Cγ1, Cγ2b and Cγ2a region set, and/or the ε heavy chain constant region) or murine light chain constant regions, preferably $C_L\kappa$ or $C_L\lambda$, by a human constant region (see paragraph [0014]).

24. Document (6) does not specifically teach or even suggest to replace the murine constant region CH1-upper hinge gene segment by the human counterpart, while retaining the murine CH2-CH3 gene segment. Thus, a person skilled in the art starting from document (4) could not arrive at the claimed invention by applying the teachings of document (6).

25. Document (5) describes expression vectors used for screening of mouse Fab libraries. The individual clones encode a hybrid antibody constant region that includes murine and human constant regions; in particular, a vector with partial human heavy chain constant region CH1 plus partial human hinge region is described (see paragraph [0029]).

26. As apparent from paragraphs [0004] and [0005] of document (5), the problem addressed by the vectors described therein, namely the relatively poor expression of murine antibodies in a host cell like,

e.g., *E. coli* compared to the level of expression of a similar human-derived antibody, clearly differs from the problem solved by the present invention. The use of such expression vectors is said to greatly increase expression compared to the expression of murine Fabs that include a fully murine constant region (see second sentence in paragraph [0022]). Hence, the board fails to see why a person skilled in the art trying to solve the problem of retaining affinity and potency when chimeric human-murine antibodies are further humanized, would have considered the teaching in document (5) of a hybrid human-murine constant region.

27. Documents (10) and (11) are scientific articles published by the same research group which provide experimental data supporting the hypothesis that the constant region may affect the secondary structure of the antigen binding site, thus accounting for variations in specificity of the antibody (see Abstract of document (10)). Particular attention is given to two regions of the CH1 domain because of their ability to form hydrogen bonds and, consequently, affect the segmental flexibility of the antibody (see paragraph bridging pages 13924 and 13925 of document (10)).

28. The board shares the appellant's view that neither document (10) nor document (11) provides a clear pointer to the particular chimeric locus defined in claim 1. Even if the skilled person, in view of the teachings of these documents, might have considered replacing the murine CH1 domain by the human counterpart, he/she would not have any reasonable expectation of success in providing human-murine chimeric antibodies that, after being fully humanized, retain the specificity and potency of the parental antibody.

29. In view of the above, the board concludes that the mouse cell of claim 1 involves an inventive step. The same applies to the method of producing it (claim 6), a knock-in mouse obtainable from the cell (claim 7), chimeric antibodies produced by the cell (claim 9) and their uses (claims 11 to 13).

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the examining division with the order to grant a patent on the basis of claims 1 to 13 of the main request filed at the oral proceedings on 25 January 2021, and a description to be adapted.

The Registrar: The Chairman:

L. Malécot-Grob B. Stolz

Decision electronically authenticated